Monitor: molecules and profiles

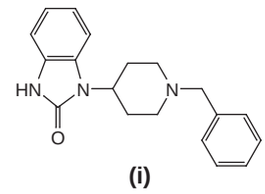
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

Molecules

Opioid receptor-like antagonist

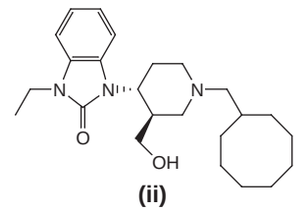
The opioid receptor, opioid receptor-like 1 (ORL-1) was identified using cloning techniques in 1994. This is the fourth receptor to be identified, and is a G-protein coupled receptor that has significant sequence homology with the classical opioid receptors (μ, δ and κ). In common with the other opioid receptors, it is coupled to activation of inwardly rectifying potassium channels and/or is negatively coupled to adenylyl cyclase. However, none of the traditional opioid receptor ligands show affinity for ORL-1. An endogeneous 17 amino acid ORL-1 ligand (nociceptin/orphanin FQ) has been identified in the brain. Although this has homology with the opioid peptide dynorphin, it has no significant activity at the other opioid receptors. Studies using ORL-1 and nociceptin/orphanin FQ-deficient mice show that this receptor has several roles including the regulation of pain response, learning and memory, food intake, anxiety and locomotor activity. Until now, further pharmacological evaluation of the role of this receptor has been limited by the lack of a suitable antagonist.

The first potent and selective small-molecule ORL-1-receptor antagonist has



(i)

been reported recently by Kawamoto, H. and coworkers[1]. The lead compound, 1-(1-benzyl-4-piperidyl)-1,3-dihydro-2*H*-benzimidazol-2-one (**i**), was identified from screening of a chemical library. This compound showed high affinity for ORL-1 (IC_{50} = 200 nM) but limited selectivity for ORL-1 over μ and κ receptors. Structural modification of this lead structure led to the identification of 1-[(3*R*,4*R*)-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one [J113397, (**ii**)] as a potent (IC_{50} = 2.3 nM) and selective ORL-1 antagonists. This compound has >300-fold selectivity for ORL-1 over the other opioid receptors and inhibits ORL-1 function.



(ii)

This compound will therefore be a useful tool for the further evaluation of the pharmacological role of ORL-1 and therapeutic value of ORL-1 agonists and antagonists.

1 Kawamoto, H. *et al.* (1999) Discovery of the first potent and selective small molecule opioid-like (ORL1) antagonist: 1-[(3*R*,4*R*)-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J113397). *J. Med. Chem.* 42, 5061–5063

Orally active GPIIb/IIIa-receptor antagonists

Fibrinogen plays a key role in the aggregation of platelets associated with serious cardiovascular disorders that lead to thrombus formation. This endogenous molecule binds to the activated, membrane-bound glycoprotein (GP) IIb/IIIa –integrin complex. Recently, various fibrinogen-receptor antagonists have been developed and commercialized as antithrombotic agents. Although these agents are effective for acute treatment of cardiovascular disorders, an orally active fibrinogen-receptor antagonist would offer the opportunity to provide a chronic regimen for prophylactic treatment of high-risk patients. Oral bioavailability of existing GPIIb/IIIa-receptor

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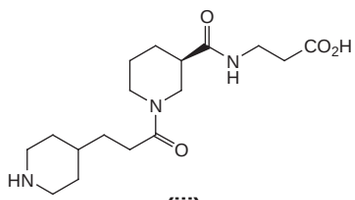
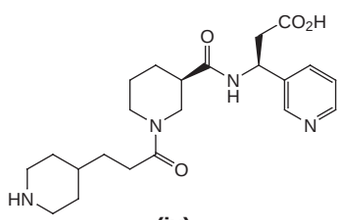
antagonists is limited by the zwitterionic nature of these nonpeptide analogues based on the integrin tripeptide recognition motif Arg-Gly-Asp (RGD) present on the Aα-chain of fibrinogen. Although various groups have attempted to overcome the problems of poor oral bioavailability using prodrugs, the use of RGD-mimetics could be limited by the presence of this sequence in a wide range of proteins.

Workers from the R.W. Johnson Pharmaceutical Research Institute (Spring House, PA, USA) have adopted an alternative strategy[2], focussing on KQAGD-mimetics. This sequence is found in the γ-chain of fibrinogen and is involved in the binding of fibrinogen to GPIIb/IIIa. Using a nipecotic acid scaffold, compound (**iii**) was initially identified as an orally active fibrinogen-receptor antagonist with modest potency and



(iii)

short duration of action. A solid-phase parallel synthesis optimization programme, involving the synthesis and evaluation of almost 250 analogues, led to the identification of RWJ53308 (**iv**) as a potent, orally active GPIIb/IIIa-receptor antagonist. In addition to demonstrating significant *ex vivo* antiplatelet activity, this compound has 16% oral bioavailability on administration at 1 mg kg^{-1} to dogs. Furthermore, this agent is efficacious in canine



(iv)

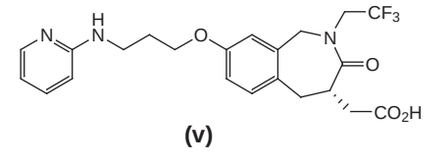
arteriovenous shunt, guinea pig photoactivation-induced injury and guinea pig ferric chloride-induced injury in thrombosis models *in vivo*. On this basis, the compound was selected for clinical evaluation as both an orally and intravenously administered antithrombotic agent, and has now successfully completed Phase II clinical trials. Clearly, compounds of this nature will be useful for both acute and chronic antiplatelet therapy in humans.

2 Hoekstra, W.J. *et al.* (1999) Potent, orally active GPIIb/IIIa antagonists containing a nipecotic acid subunit. Structure–activity studies leading to the discovery of RWJ-53308. *J. Med. Chem.* 42, 5254–5265

Integrin $\alpha_v\beta_3$-receptor antagonists

The vitronectin receptor $\alpha_v\beta_3$ is a member of the integrin family of transmembrane heterodimeric glycoprotein complexes. This integrin is expressed on most cells of mesenchyme origin and plays a role in many biological processes including osteoclast adhesion to bone matrix, vascular smooth muscle cell migration and angiogenesis. Hence, there has been significant interest in the development of integrin $\alpha_v\beta_3$-receptor antagonists for the treatment of several diseases including osteoporosis, restinosis, rheumatoid arthritis, cancer and angiogenic ocular disorders. Like the GPIIb/IIIa–integrin complex already described, the $\alpha_v\beta_3$ integrin binds to a range of RGD-containing adhesion proteins including vitronectin, fibrinogen, osteopontin, fibronectin and von Willebrand factor. Various groups have therefore focussed their research on the development of RGD-peptidomimetics as potential $\alpha_v\beta_3$-receptor antagonists.

In a recent communication, workers from SmithKline Beecham Pharmaceuticals (Collegeville, PA, USA) have described the identification of a new class of potent and orally bioavailable $\alpha_v\beta_3$-receptor antagonists based on a 2-benzazepine Gly–Asp scaffold[3]. In biological studies, compounds (**v**) and (**vi**) inhibited osteoclast-mediated bone resorption *in vitro* (IC_{50} = 29 nM and 11 nM, respectively). Both compounds



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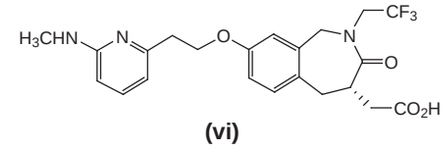
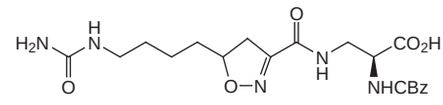


(vi)

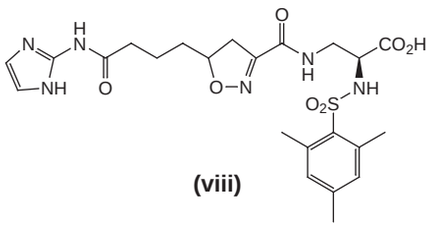
were also active in the *in vivo* thyroid- and parathyroid-ectomized rat model of bone resorption (EC_{50} = 35 μM and 20 μM, respectively) and orally active in the ovariectomized rat model of osteoporosis. Hence, these and similar agents might be therapeutically useful in the treatment of osteoporosis in humans.

Two other recent reports from workers at DuPont Pharmaceuticals Company (Wilmington, DE, USA) describe the identification of isoxazolines and disubstituted indazoles as potent $\alpha_v\beta_3$-receptor antagonists[4,5].



(vii)

Previous studies by workers from this company led to the identification of (**vii**) as a potent inhibitor of vitronectin through binding to the purified $\alpha_v\beta_3$ receptor. However, this agent showed poor selectivity for this receptor over GPIIb/IIIa receptors. Structural optimization led to the identification of compounds (**viii**) and (**ix**) possessing nearly 1000-fold greater selectivity for the $\alpha_v\beta_3$ receptor over the GPIIb/IIIa receptor. In addition, the aminoimidazolylurea (**ix**) inhibited vitronectin- stimulated, $\alpha_v\beta_3$-mediated cell migration.



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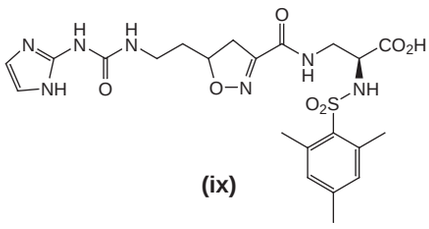
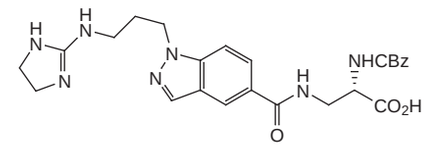


(ix)

In a separate paper, workers from the same company have described the optimization of the lead indazole (**x**) to yield compound (**xi**), which is a potent $\alpha_v\beta_3$-receptor antagonist (IC_{50} = 2.3 nM) with a ninefold selectivity over GPIIb/IIIa.



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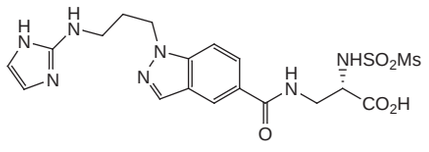
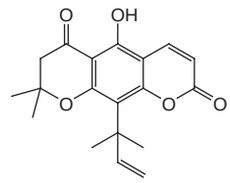


(xi)

1,1-Dimethylallylcoumarins as nitric oxide generation suppressors

The free radical nitric oxide is involved in several diseases including inflammation and carcinogenesis. It has been suggested that certain natural products prevent the occurrence of these diseases by limiting nitric oxide generation. For example, previous studies have shown that coumarins isolated from *Citrus hystrix* DC fruit inhibit both lipopolysaccharide- and interferon γ-induced nitric oxide production in mouse macrophage RAW 264.7 cells.

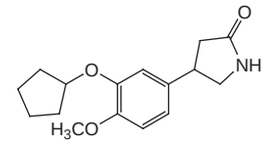
In a recent paper, Murakami, A. and coworkers have described the screening of 16 coumarin-related compounds on both lipopolysaccharide- and interferon γ-induced nitric oxide production using this model[6]. Coumarins with prenyl unit(s) were found to be highly effective at reducing nitric oxide generation. The most effective compounds tested were the 1,1-dimethylallylcoumarins, exemplified by clausenidin (**xii**). Western blotting demonstrated that these compounds inhibit nitric oxide generation by suppressing the expression of inducible nitric oxide synthase. This finding might have important implications for the future development of novel nitric oxide generation suppressants.



(xii)

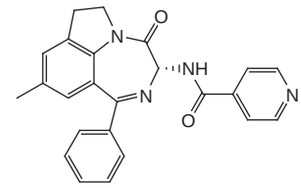
Novel PDE4 inhibitors

Phosphodiesterase 4 (PDE4) participates in the cellular regulation of cAMP in allergenic diseases. Specific inhibitors of this enzyme have therefore been sought for the potential treatment of diseases such as asthma. Most existing inhibitors are based on Rolipram (**xiii**) and exhibit CNS side effects such as emesis.



(xiii)

A recent paper from Pascal, Y. and coworkers describes the synthesis and evaluation of several novel benzodiazepine derivatives as PDE4 inhibitors[7]. These compounds show selectivity for PDE4 over other phosphodiesterases.



(xiv)

The *in vivo* activity of these compounds was exemplified by compound (**xiv**), which can inhibit eosinophil infiltration in sensitized Brown–Norway rats at 5.1 mg kg^{-1} orally. Furthermore, no emetic side effects were observed on intravenous administration of 3 mg kg^{-1} to rats. This family of compounds might offer useful leads for the future development of more potent, specific and orally active PDE4 inhibitors.

3 Miller, W.H. *et al.* (2000) Discovery of orally active nonpeptide vitronectin receptor antagonists based on a 2-benzazepine Gly–Asp mimetic. *J. Med. Chem.* 43, 22–25

4 Pitts, W.J. *et al.* (2000) Isoazolines as potent antagonists of the integrin $\alpha_v\beta_3$. *J. Med. Chem.* 43, 27–40

5 Batt, D.G. *et al.* (2000) Disubstituted indazoles as potent antagonists of the integrin $\alpha_v\beta_3$. *J. Med. Chem.* 43, 41–58

6 Murakami, A. *et al.* (2000) 1,1-Dimethylallylcoumarins potently suppress both lipopolysaccharide- and interferon-γ-induced nitric oxide generation in mouse macrophage RAW 264.7 cells. *Bioorg. Med. Chem. Lett.* 10, 59–62

7 Pascal, Y. *et al.* (2000) Synthesis and structure–activity relationships of 4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indoles: Novel PDE4 inhibitors. *Bioorg. Med. Chem. Lett.* 10, 35–38

Andrew Lloyd

Emerging molecular targets

Exploiting nutraceutical treatments for osteoarthritis and ischaemia

The regulation of the nutraceutical industry has received significant attention from lobbying organizations such as the American Association for Pharmaceutical Scientists over recent years. A recent review on the therapeutic nutraceutical treatments for osteoarthritis and ischaemia by Grant, G.F. and Gracy, R.W. illustrates the potential therapeutic value of these products[1].

The review focusses on nutraceuticals that share common biochemical pathways such as glucosamine, ribose and their derivatives. It would appear that in aged individuals, the cellular regulation of the hexose monophosphate pool limits the production of cellular energy and cartilage in various tissues. The administration of ribose and glucosamine circumvents this regulation through their direct involvement in biochemical pathways for cellular energy maintenance and the repair of cartilage and connective tissues, respectively, in active middle-aged individuals. Oral ribose restores cellular energy lowered by ischaemia that occurs following, for example, myocardial infarction. Meanwhile, oral glucosamine alleviates the symptoms of osteoarthritis by stimulating the synthesis of glycoaminoglycans (GAGs) and thereby facilitating the repair of cartilage and connective tissue. The review concludes by suggesting that, in the future, the success of patented nutraceuticals in the treatment of osteoarthritis and ischaemia will facilitate targeted pharmaceutical intervention to overcome the rate-limiting pathways involved in GAG and ATP synthesis.

The demand for supplements containing these agents will undoubtedly rise with increasing average life expectancy. Furthermore, future evaluation of other biochemical pathways will undeniably lead to the identification of other supplements that might overcome biochemical regulation of cellular pathways in the aged individual, offering improved quality and longevity of life.

1 Grant, G.F. and Gracy, R.W. (2000) Therapeutic nutraceutical treatments for osteoarthritis and ischaemia. *Exp. Opin. Ther. Patents* 10, 39–48

Andrew Lloyd

Combinatorial chemistry

Small-molecule–RNA interactions

The interactions between proteins and nucleic acids are crucial to many biological functions including transcription, RNA splicing and translation. The ability to design selective and potent small compounds that can bind to RNA and DNA is a significant step in the discovery of novel drug molecules. A recent study describes the use of encoded combinatorial libraries in the discovery of novel anti-HIV-1 agents[1].

The transcriptional upregulation of HIV-1 gene expression depends on the binding of the TAT protein to the transactivation response region (TAR) RNA. This is a 59-base stem-loop sequence at the 5′-end of all nascent HIV-1 transcripts. An encoded library of 24,839 possible tripeptide sequences synthesized using all D- and L-alpha amino acids has been prepared on TentaGel resin and incubated with disperse red-labelled TAR. Beads that became red or pink were sequenced by analysis of the encoding molecules by electron-capture gas chromatography.

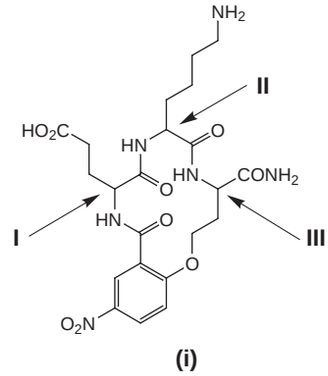
The two most potent tripeptide sequences discovered were (L)Lys-(D)Lys-(L)Asn and (D)Thr-(D)Lys-(L)Asn, suggesting a consensus sequence of X-(D)Lys-(L)Asn. Further analysis revealed that diastereoisomers of the first ligand were much weaker binders indicating that the interaction with TAR is highly stereospecific, and not merely the result of a non-specific Lys– Phosphate interaction. (L)Lys-(D)Lys-(L)Asn was shown to suppress transcriptional activation by the TAT protein in human cells with an IC_{50} value of approximately 50 nM.

1 Hwang, S. *et al.* (1999) Inhibition of gene expression in human cells through small molecule–RNA interactions. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12997–13002

Stereochemical diversity

The focus on diversity in combinatorial libraries is primarily on factors such as MW, lipophilicity, the numbers and types of hydrogen bonds, and the presence of key pharmacophores. However, stereochemistry and conformation also contribute considerably to the assessment of diversity and have been the subject of a recent publication[2].

To prepare libraries of peptidomimetic turn-mimics, targeted for example at nerve growth factor (NGF), it became apparent that conformational diversity would be maximized by the incorporation of D-amino acids. As D-amino acids might be more expensive to use than L-amino acids, compounds that varied the I, II and III positions of compound (**i**) were investigated to explore the impact on conformational diversity. Using circular dichroism and NMR, the overall conclusion was that stereochemical variation of the II position led to the greatest effects



(i)

on conformational diversity.

2 Feng, Y. *et al.* (1999) Stereochemical implications on diversity in β-turn peptidomimetic libraries. *J. Org. Chem.*

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